Spermiogenesis and Modified Sperm Morphology in the "Seepworm" *Methanoaricia dendrobranchiata* (Polychaeta: Orbiniidae) From a Methane Seep Environment in the Gulf of Mexico: Implications for Fertilization Biology

KEVIN J. ECKELBARGER[1] AND CRAIG M. YOUNG[2]

[1]*Darling Marine Center, 193 Clark's Cove Road, Walpole, Maine 04573; and* [2]*Division of Marine Sciences, Harbor Branch Oceanographic Institution, 5600 U.S. 1 North, Ft. Pierce, Florida 34946*

Abstract. Spermatogenesis and mature sperm morphology have been described along with limited observations of the ovary in *Methanoaricia dendrobranchiata,* an orbiniid polychaete associated with dense populations of the mussel *Bathymodiolus childressi* at brine pools on the Louisiana slope, Gulf of Mexico. The species is gonochoric with gonads serially repeated in numerous segments and each associated with a nexus of blood vessels at the base of the parapodia. In the female, synchronous, intraovarian egg development occurs with the release from the ovary of large, yolky eggs into the coelom at first meiotic metaphase. Sperm develop in the coelom as free-floating, plasmodial clones interconnected *via* an anuclear cytophore. At the end of spermiogenesis, mature spermatozoa float freely in the coelom. The mature spermatozoon differs significantly from that of shallow-water orbiniid species by possessing an elongated nucleus and a greatly elongated and curved acrosome reaching 19.5 μm in length. The spermatozoon resembles an ent-aquasperm and may not fertilize the eggs directly in seawater in the classical manner. We hypothesize that the unusual spermatozoon morphology in this species has evolved due to the hypoxic environment in which the adults live and that fertilization biology is likely modified in some way to minimize sperm exposure to high levels of hydrogen sulfide. An analysis of life-history features in shallow-water orbiniids is used to infer reproductive features in *M. dendrobranchiata* that could not be directly documented.

Introduction

The reproductive biology of invertebrates from deep-sea hydrothermal vents and cold methane seeps continues to receive a great deal of attention (Tyler and Young, 1999). Hydrothermal vents and cold-water methane seep habitats show significant ecological differences, and the two kinds of communities have few species in common (Tunnicliffe, 1991). There are, however, strong taxonomic affinities between seeps and vents, suggesting that divergent life-history features could evolve in closely related species. Studies on deep-sea polychaetes have been almost exclusively directed at hydrothermal vent species (reviewed in Eckelbarger *et al.,* 2001) and have included only 3 of 80 polychaete families. Polychaetes show a remarkable diversity of reproductive attributes and life-history features (Schroeder and Hermans, 1975; Wilson, 1991) and offer abundant opportunities to study the evolution of reproductive mechanisms. Despite the ecological importance of this group, the entire life history is known for only about 3% of known species (Giangrande, 1997). Recently, Eckelbarger *et al.* (2001) described the reproductive biology of the hesionid polychaete *Hesiocaeca methanicola,* the first such description for any methane-seep polychaete. A recent series of papers describes the reproductive biology of other methane-seep invertebrates from the Gulf of Mexico, including oogenesis (Eckelbarger and Young, 1997) and spermatogenesis (Hodgson *et al.,* 1998) in the neritid gastropod *Bathynerita naticoidea,* and gametogenesis in the seep mussel *Bathymodiolus childressi* (Eckelbarger and Young, 1999).

In contrast to studies of shallow-water invertebrates, studies of the reproductive biology of deep-sea species can be challenging, especially direct observations of spawning and fertilization. However, sperm ultrastructure can have predictive value in indirectly assessing possible fertilization mechanisms, because sperm morphology is highly correlated with the biology of fertilization (Franzen, 1956). Sperm structure is also widely used in the analysis of phylogenetic relationships within the Polychaeta (Rouse, 1995). It has been specifically noted that sperm ultrastructure from species occupying deep-sea reducing environments can be particularly revealing when compared to spermatozoa of related taxa from more conventional habitats (Beninger and Le Pennec, 1997).

During several submersible dives to the brine-pool methane seep at 650 m in the Gulf of Mexico, we encountered dense populations of a robust, unidentified polychaete living among individuals of *Bathymodiolus childressi*, a chemosynthetic mytilid bivalve, ringing a pool of hypersaline brine. The worms were observed extending the anterior portions of their bodies above the surrounding mussels into the overlying water, an unusual behavior in polychaetes. Low oxygen and high sulfide levels have been reported in this habitat (Smith *et al.*, 2000) and it is believed that the worms are subject to respiratory stress (Hourdez *et al.*, 2001). This specific hydrocarbon seep site was described previously by MacDonald *et al.* (1990) and MacDonald (1998). MacDonald *et al.* (1990) noted the presence of an "unidentified paranoid [*sic*] polychaete". Blake (2000) later described it as a new genus and species of orbiniid polychaete, *Methanoaricia dendrobranchiata* Blake 2000, and suggested that its unusual adult morphology may reflect adaptations to a hypoxic environment. A recent study concluded that this species differs from other orbiniids in several ways, including the presence of anterior hypertrophied gills (Hourdez *et al.*, 2001) that may be required in its oxygen-limited habitat. In our preliminary examination of the sperm of *M. dendrobranchiata*, we noted that the sperm heads were extremely long and sickle-shaped, an uncommon sperm morphology for any polychaete. In this paper, we describe the ultrastructural features of spermatogenesis and mature sperm morphology in *M. dendrobranchiata* and speculate on the phylogenetic and life-history significance of its occurrence in this unusual marine habitat.

Materials and Methods

Living specimens of *Methanoaricia dendrobranchiata* were collected in August 1997 by the *Johnson-Sea-Link II* submersible from the "brine pool" at Green Canyon, MMS Block 232, in the Gulf of Mexico (MacDonald *et al.*, 1990) at 650-m depth (27°43.327'N, 91°16.606'W). The worms were found in dense mussel beds (*Bathymodiolus childressi*) within 1 m of the pool perimeter. Specimens were

brought to the surface in carousel buckets on the submersible and immediately transferred to glass containers containing cold (8°C) filtered (45 μm) seawater in the walk-in cold room of the ship. The sex of specimens was determined by puncturing the body wall with a fine needle and examining the extruded coelomic contents under a compound microscope for the presence of sperm or eggs. Four females and ten males of varying lengths (10–15 cm) were selected for fixation. A razor blade was used to cut cross sections of 2–4 body segments containing coelomic sperm or ovaries. The tissue samples were immersed for 1.5 h in cold (4°C) primary fixative (2.5% glutaraldehyde buffered with 0.2 M sodium phosphate), and post-fixed for 1.5 h in room temperature 1% osmium textroxide buffered with 1.25% sodium bicarbonate. Tissue was then dehydrated in ascending concentrations of ethanol to 100%, followed by two changes with propylene oxide (3 min each), and embedded in Epon. Only thick sections were cut of females, but thin sections of males were cut with a diamond knife on a Porter-Blum MT2-B ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Zeiss EM 10A transmission electron microscope.

Results

Female

Methanoaricia dendrobranchiata is a gonochoric species, but the sexes cannot be distinguished externally. Although sperm development is the primary subject of this paper, we also made preliminary observations on female specimens, examining thick sections with a light microscope. Pairs of serially repeated ovaries are found in most segments and are associated with a nexus of blind-ending genital blood vessels at the base of the parapodia. Individual oocytes lie close to the blood vessels (Fig. 1), and thin follicle cells surround each oocyte. Oogenesis is intraovarian; developing oocytes remain within the ovary until at least the late stages of vitellogenesis, when they are released into the coelom. Sexually mature females were so packed with eggs that the coelomic spaces were nearly obliterated. In the specimens we examined, oogenesis was synchronous; the majority of oocytes were in late stages of vitellogenesis, although a few previtellogenic oocytes were observed. Most intraovarian oocytes contained a prominent germinal vesicle with a single eccentric nucleolus and relatively large amounts of lipid-type yolk stores (Fig. 2). Coelomic eggs measured about 280 μm, and all female specimens we examined showed evidence of germinal vesicle breakdown (Figs. 3, 4) indicating that prematuration has occurred and the primary oocytes have proceeded to first meiotic metaphase.

Male

Germ cells enter the coelom in the early stages of spermatogenesis, and in all specimens examined, the coelomic
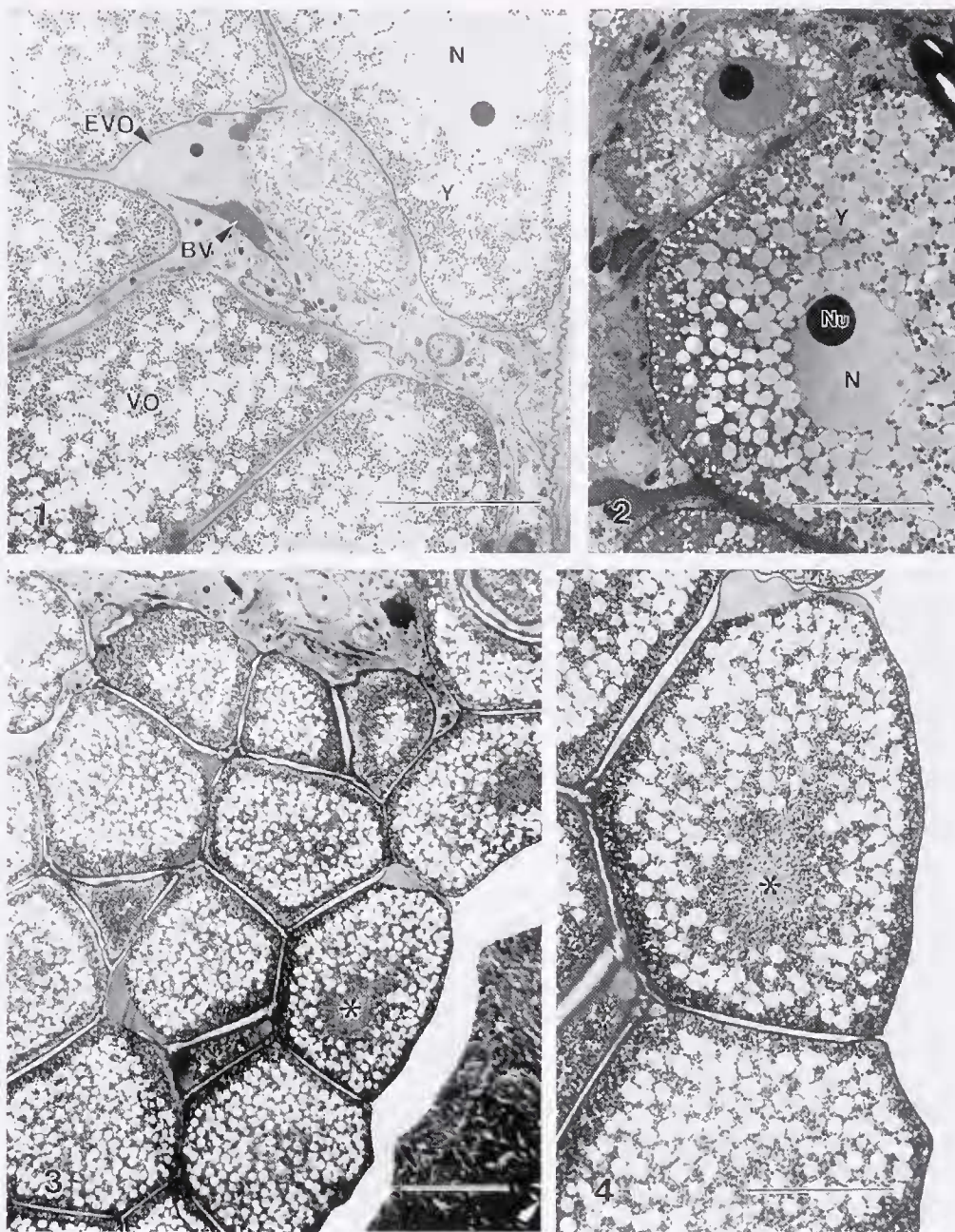
Figure 1. Light micrograph of ovary showing blood vessel (BV) surrounded by early vitellogenic oocyte (EVO) and large, vitellogenic oocytes (VO). N = nucleus; Y = yolk granule. Scale = 100 μm.
Figure 2. Close-up of oocyte containing nucleus (N), nucleolus (Nu), and yolk granules (Y). Scale = 75 μm.
Figure 3. Coelomic oocytes showing apparent germinal vesicle breakdown (*). Scale = 140 μm.
Figure 4. Close-up of coelomic oocyte showing germinal vesicle breakdown (*). Scale = 120 μm.

cavity was tightly packed with gametes in all stages of development (Fig. 5), including single, free-floating mature sperm (Figs. 5, 6). Sperm develop as disc-shaped, plasmodial clones (=sperm morulae) 30–40 μm in diameter consisting of 50–70 cells interconnected to a central, anucleate cytophore whose origin is unknown (Fig. 8). Gametogenic

stages undergo synchronous development and remain associated with the morulae until late spermiogenesis, when mature sperm apparently detach and float individually in the coelomic fluid. Mitotic and meiotic cell divisions are readily apparent in histological section.

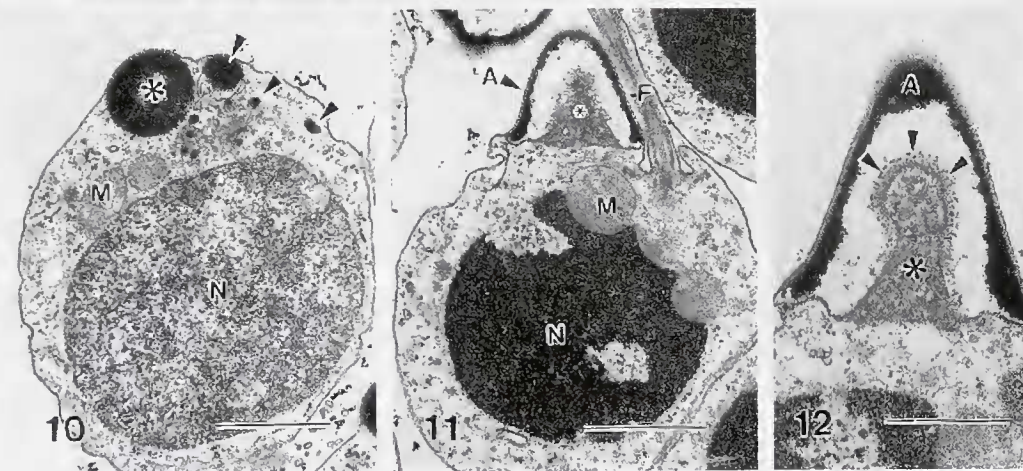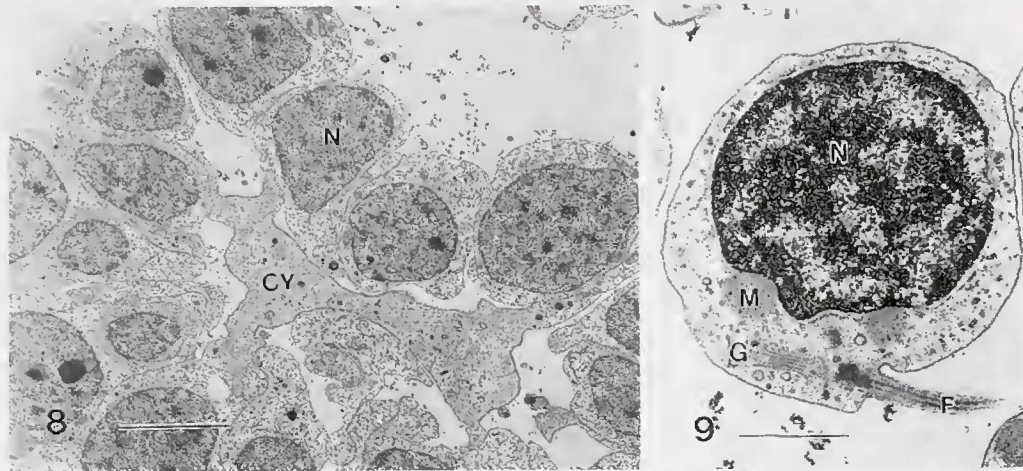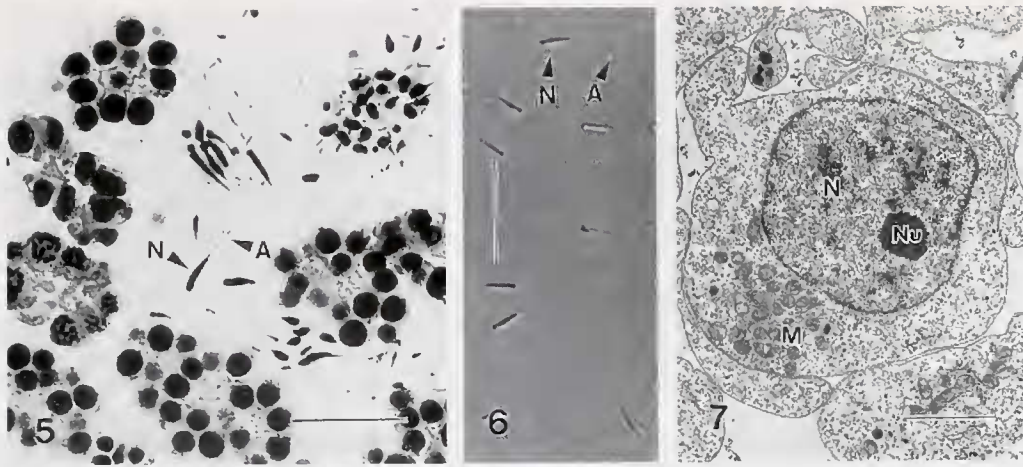Spermatocytes are spherical cells about 8.0 μm in

Figure 5. Light micrograph showing sperm and sperm morulae floating in coelom. The nucleus (N) and acrosome (A) of the coelomic sperm are evident. Scale = 20 μm.

Figure 6. Light micrograph of living sperm showing elongated nucleus (N) and acrosome (A). Scale = 20 μm.

Figure 7. Spermatocyte containing nucleus (N), nucleolus (Nu), and clustered mitochondria (M). Scale = 2.0 μm.

Figure 8. Sperm morula with central cytophore (CY) and attached spermatocytes with large nuclei (N). Scale = 4.0 μm.

Figure 9. Spermatid containing nucleus (N), mitochondria (M), Golgi complex (G), and flagellum (F). Scale = 2.0 μm.

Figure 10. Spermatid showing large (*) proacrosomal granule and adjacent smaller ones (arrowheads), mitochondria (M), and nucleus (N). Scale = 2.2 μm.

Figure 11. Spermatid showing acrosomal vesicle (A) with subacrosomal material (*). N, nucleus; M, mitochondrion; F, flagellum. Scale = 2.0 μm.

Figure 12. Spermatid acrosomal vesicle (A) with growing subacrosomal region consisting of an electron-dense outer region (arrowheads) and flocullent interior (*). Scale = 1.0 μm.

diameter containing large nuclei (dia. 5.5 μm) with finely diffuse heterochromatin and a single nucleolus (Figs. 7, 8). Their cytoplasm is devoid of organelles except for clusters of small perinuclear mitochondria (Fig. 7). Early spermatids have spherical nuclei (6.5 μm dia.) with lightly staining heterochromatin and small mitochondria positioned in shallow nuclear fossae at the future posterior pole of the cell (Fig. 9). A single Golgi complex is associated with a developing flagellum that projects at a 90° angle from the future anterior-posterior axis. The cytoplasm of early spermatids often contains a number of proacrosomal granules (Fig. 10) that later coalesce into a single organelle. As spermiogenesis progresses, chromatin condensation results in moderately dense, spherical nuclei (4.5 μm dia). A small cone-shaped acrosomal vesicle projects from the cell surface in close association with the flagellum and associated distal centriole (Fig. 11). As the acrosomal vesicle elongates, it tapers to a blunt tip and retains an electron dense appearance for approximately the anterior two-thirds of its length while the basal region contains a light staining flocculent subacrosomal material (Fig. 12). The elongating acrosomal vesicle remains at the posterior end of the cell until the late stages of spermiogenesis (Figs. 13–15), when it abruptly appears in the anterior region (Fig. 16). As it elongates, the subacrosomal component is clearly visible in the posterior region as a digitate, central projection that appears hollow in the anterior portion (Fig. 17). The flagellum still projects from the posterior region of the cell nearly 90° from the longitudinal axis (Fig. 18).

The mature spermatozoon has a small midpiece, an elongate, bullet-shaped nucleus 6.5 μm in length with a bulbous base and a gently curved anterior surface, and a gently curved, sickle-shaped acrosome about 19.5 μm in length, giving the sperm head a total length of about 26 μm (Figs. 19, 20). The acrosomal vesicle flares gently at its base (Fig. 21) and gradually tapers to a relatively blunt tip. Internally, the posterior region of the acrosome contains a solid cone-shaped mass of flocculent subacrosomal material that extends anteriorly within the acrosomal vesicle (Figs. 21–24). The subacrosomal material contains a centrally positioned rod-like structure that is more electron dense than the surrounding material and that projects anteriorly about 0.5 μm (Fig. 23). In the more anterior region of the acrosome, the subacrosomal material has a more filament-like substructure (Fig. 24). The midpiece contains six mitochondria positioned within shallow nuclear fossae (Fig. 28), and a centriolar complex and associated flagellum. In cross section, the distal centriole possesses radiating, satellite rays that extend to the adjacent cell plasmalemma and trifurcate to form a dense ring (Fig. 26). Whereas the distal centriole lies parallel with the longitudinal axis of the cell, the proximal centriole is laterally positioned at a 30° angle (Fig. 25). The two centrioles are joined by a flocculent satellite connector (Fig. 27).

We saw no evidence that mature sperm are concentrated in a particular region of the body prior to spawning (as, for example, in a nephridium), and we did not observe a seminal vesicle in any male specimen.

Discussion

Orbiniid polychaetes are common infaunal residents of bays and estuaries throughout the world (Hartman, 1957) but are also represented in deep-sea hydrothermal vent communities (Tunnicliffe *et al.*, 1998). Limited information is available on the reproductive biology of shallow-water species (Anderson, 1961; Blake, 1980; Giangrande, 1997) (Table 1) and nothing is known about those from deep-water habitats. Orbiniids show a high degree of conservatism with respect to life-history features including annual, iteroparous breeding patterns; deposition of relatively large eggs in jelly masses or cocoons; lecithotrophic or nonpelagic development (Chapman, 1965; Anderson, 1961; Blake, 1980; Giangrande, 1997); and ect-aquasperm (*sensu* Rouse and Jamieson, 1987). On the basis of a study of *Nainereis laevigata* (Giangrande and Petraroli, 1991), our observations of *Methanoaricia dendrobranchiata* (present study), and previous studies of *Leitoscoloplos fragilis* (Eckelbarger, pers. obs.), we conclude that intraovarian oogenesis and ovaries associated with blood vessels are probably additional family traits.

Little is known about orbiniid spermatogenesis and sperm morphology except for a light microscopic study on *Nainereis laevigata* by Giangrande and Petraroli (1991), in which they reported developing coelomic sperm "platelets." Rice (1992) made some ultrastructural observations of mature sperm morphology for *Leitoscoloplos pugettensis* (=*Leitoscoloplos elongatus*). Both species have sperm of the ect-aquasperm type found in broadcast-spawning species; characteristics include a rounded head with a simple cap-like acrosome, four or five spherical mitochondria in the midpiece, and a tail with 9 + 2 arrangement of microtubules (Franzen, 1956). Rice (1992) noted that *Leitoscoloplos pugettensis* also possesses an eccentric flagellum, but we could not confirm this in *M. dendrobranchiata*. Ect-aquasperm are the most common sperm in the Polychaeta and are considered plesiomorphic for the group (Jamieson and Rouse, 1989). The presence of intracoelomic sperm morulae in both *M. dendrobranchiata* and *N. laevigata* suggests that this form of sperm differentiation is also a plesiomorphic feature. The shape of sperm morulae and the number of sperm they contain have proven useful characters for phylogenetic analysis (Rouse and Fitzhugh, 1994; Rouse, 1995).

The sperm head (nucleus plus acrosome) of *Methanoaricia dendrobranchiata* is curved, needle-like, and extraordinarily long, with the acrosome accounting for about two-thirds of the head length. The acrosome is among the

Figure 13. Spermatid with elongating acrosomal vesicle (A) and small subacrosomal material (arrowhead). N, nucleus; M, mitochondrion; G, Golgi complex. Scale = 2.0 μm.

Figure 14. Spermatid with acrosomal vesicle (A), subacrosomal material (*), flagellum (F) with associated centriole (C), mitochondrion (M), and nucleus (N). Scale = 2.0 μm.

Figure 15. Spermatid with acrosomal vesicle (A) and subacrosomal material (*). N, nucleus; M, mitochondrion. Scale = 2.0 μm.

Figure 16. Spermatid with acrosomal vesicle (A) and elongated subacrosomal material (*). N, nucleus. Scale = 2.0 μm.

Figure 17. Closeup of Fig. 16 showing features of subacrosomal (SA) region of acrosomal vesicle (A). Arrowheads indicate fuzzy external layer; note hollow anterior region (*). Scale = 1.0 μm.

Figure 18. Middlepiece region of sperm showing nucleus (N), mitochondria (M), and flagellum (F) and associated distal centriole (DC) that projects at a 90° angle. Scale = 2.0 μm.

Figure 19. Mature spermatozoon showing elongated acrosome (A) and nucleus (N). Insert shows transverse section through anterior region of acrosome. Scale = 3.0 μm.

Figure 20. Nuclear (N) region of spermatozoon showing grazing section of anterior acrosomal vesicle (A). M, mitochondrion. Scale = 2.0 μm.

Figure 21. Posterior region of acrosomal vesicle (A) showing subacrosomal space consisting of dense basal plate (arrowheads) and flocculent interior (*). Scale = 1.5 μm.

Figure 22. Transverse section through region where acrosomal vesicle (A) meets the anterior nucleus (N) (see Fig. 21). Scale = 1.5 μm.

Figure 23. Posterior region of acrosomal vesicle (A) showing details of subacrosomal area including dense basal plate and digitate process in posterior region (arrowheads), and flocculent central region further anteriorly (*). N, nucleus. Insert shows transverse section through anterior acrosomal vesicle. Scale = 1.5 μm.

Figure 24. Anterior region of acrosomal vesicle (A) showing filamentous interior (*). Insert shows transverse section through vesicle. Scale = 1.5 μm.

Table 1

Life-history traits of the Orbiniidae

Species	Periodicity	Egg Dia.	Pelagic Phase	Egg Mass	Citation
Leitoscoloplos robustus	?	160	3.5 days	No	Horn & Bookhout (1950)
Leitoscoloplos kerguelensis	?	200	3 days	Yes	Okuda (1946)
Leitoscoloplos pugettensis	?	210	omitted or 1–9 days	Yes	Blake (1980)
Scoloplos armiger Type I	? annual	250	omitted	Yes	Anderson (1959), Gibbs (1968), Thorson (1946)
Scoloplos armiger Type II	?	230	~10 days	Yes	Sveshnikov (1960)
Scoloplos simplex	?	150	5 days	Yes	Anderson (1961)
Scoloplos acmeceps	?	188–210	~20 days	No	Blake (1980)
Phylo foetida foetida	?	?	omitted	Yes	Salensky (1883)
Phylo foetida atlantica	?	200	9 days	No	Cazaux (1972)
Naineris laevigata	?	250	1.5 days	Yes	Okuda (1946), Strathmann (1987)
" "	annual	240	?	Yes	Giangrande & Petraroli (1991)
Microrbinia linea	continuous?	200–364 × 75–152	?	?	Blake (1993)
Methanoaricia dendrobranchiata	?	~280	?	?	Present study

longest recorded for any polychaete spermatozoon and is certainly the longest for any large-bodied species. Of the 138 polychaete species for which Rice (1992) lists sperm dimensions, only 5% have acrosomes exceeding 10.0 μm— all of them from small-bodied, interstitial species in the family Protodrilidae. Protodrilid species in which fertilization occurs in the coelom of the female often transfer sperm *via* spermatophores, and they frequently have highly modified filiform introsperm (Jamieson and Rouse, 1989), one of which has an acrosome reaching 50.0 μm in length. The presence of such an unusually modified spermatozoon in *M. dendrobranchiata* is unexpected because orbiniids are traditionally considered to be broadcast spawners with simple ect-aquasperm. Ect-aquasperm have been described from three shallow-water orbiniids—*Nainereis laevigata* (Giangrande and Petraroli, 1991), *Leitoscoloplos pugettensis* (Rice, 1992), and *L. fragilis* (Eckelbarger, pers. obs.)—none of which has a modified acrosome. Blake (1993) described sperm with elongated heads measuring 8–10 μm in length from the continental slope orbiniid *Microrbinia linea*, although no details of acrosome length were noted. Large acrosomes have been reported in other polychaetes with introsperm as well as some ent-aquasperm and have been attributed to features of the egg (Jamieson and Rouse, 1989). In oligochaetes, Jamieson *et al.* (1983) clearly dem-

onstrated a statistical correlation between sperm acrosome length and thickness of the vitelline envelope. However, Jamieson and Rouse (1989), using data from Eckelbarger and Grassle (1987a, b), found a high negative correlation between sperm acrosome length and egg dimensions in capitellid polychaetes. Franzen (1983) suggests that, in bivalves, the type of nuclear elongation seen in *M. dendrobranchiata* is correlated with egg size and lecithotrophic development.

We do not believe that acrosome elongation in *Methanoaricia dendrobranchiata* sperm is correlated with attributes of the egg, because the egg closely resembles that observed in two shallow-water orbiniids, *Leitoscoloplos pugettensis* (Rice, 1992) and *L. fragilis* (Eckelbarger, pers. obs.), both of which have ect-aquasperm with a simple cap-like acrosome. *M. dendrobranchiata* is an aerobic organism that thrives in a microhabitat that is both hypoxic and highly sulfidic (Smith *et al.*, 2000; Nix *et al.*, 1995). Its anterior gills are hypertrophied, apparently to facilitate gas exchange, and its gill cells contain many mitochondria and electron-dense organelles that may play a role in sulfide detoxification (Hourdez *et al.*, 2001). We suggest that the unusual sperm morphology observed in this species is an indication that fertilization does not occur through simple broadcast spawning into seawater, but rather by some mech-

Figure 25. Midpiece of spermatozoon showing nucleus (N), mitochondria (M), distal (DC) and proximal (PC) centrioles and associated flagellum (F). Scale = 1.0 μm.

Figure 26. Pericentriolar complex of spermatozoon showing distal centriole (DC) with satellite rays (SR) attaching to dense ring-like structure (DR) and microtubules extending to the nucleus (arrowheads). Scale = 0.8 μm.

Figure 27. Proximal centriole (PC) attached to distal centriole (*) *via* a satellite connector (arrowhead). Note satellite rays (SR) projecting from distal centriole. Scale = 1.0 μm.

Figure 28. Transverse section through spermatozoon midpiece showing mitochondria (M) encircling the basal region of the nucleus (N). Scale = 1.0 μm.

anism that limits exposure of gametes to high sulfide levels. Roughly half of the orbiniid species studied to date release their eggs into jelly-coated egg masses or cocoons (reviewed by Blake, 1996), so it is possible that *M. dendrobranchiata* also protects its eggs from the sulfide environment with a jelly mass. This would probably require that the sperm penetrate a matrix in the egg mass to achieve fertilization. It is also conceivable that the jelly used to form the cocoon is chemically distinct from that used by related species in nonsulfide environments. To our knowledge, Blake (1980) is the only worker to have observed spawning in any orbiniid. He described egg release and cocoon formation in *Leitoscoloplos pugettensis* but did not observe sperm release. Although copulation (De Groot, 1907) and pseudocopulation (Anderson, 1959) have been proposed for other orbiniids, Chapman (1965) suggested that males and females come into close association during spawning and that eggs are fertilized as they emerge along with the jelly secretions. We view this as a likely scenario for *M. dendrobranchiata*. We also noted that all of the coelomic eggs in the females of *M. dendrobranchiata* that we examined had undergone germinal vesicle breakdown, indicating that the eggs had proceeded to first meiotic metaphase and were ready for fertilization. Polychaete eggs undergo fertilization either before or after germinal vesicle breakdown, and this "prematuration" event can occur before or after spawning (reviewed in Schroeder and Hermans, 1975). Intracoelomic germinal vesicle breakdown may indicate that fertilization occurs quickly after egg release as a way to minimize egg exposure to sulfides.

Until more is known about spawning behavior, we would categorize the sperm of *M. dendrobranchiata* as a putative ent-aquasperm (*sensu* Jamieson and Rouse, 1989), unlike the ect-aquasperm of other orbiniids previously described. Ent-aquasperm may swim freely in seawater at some stage or they may be stored by the female or reach the female *via* spermatozeugmata or spermatophores prior to fertilization (Rouse and Fitzhugh, 1994). They may be significantly modified from the basic plesiosperm type, and they appear in species with poorly motile females that produce large, yolky eggs (Jamieson and Rouse, 1989). For example, the sperm of the sabellid *Perkinsiana rubra* has a round nucleus but an extremely long acrosome (Chughtai, 1986). Knight-Jones and Bowden (1984) reported specimens of *P. antarctica* brooding embryos in the branchial crown. The spermatozoon of this species has been classified as an ent-aquasperm on the basis of ultrastructural criteria and the fact that it likely encounters the eggs in a jelly mass in the crown (Rouse, 1992; Rouse and Fitzhugh, 1994). We are certain that spermatozeugmata are not employed in *M. dendrobranchiata*, because mature sperm are free in the coelom prior to spawning. Sperm storage, spermatophores, or both might be employed, but we have no direct evidence of this.

Reproductive studies of deep-sea species are often diffi-

cult because seasonal sampling and direct observation of spawning behavior and fertilization may be impossible. For this reason, an analysis of reproductive and life-history features of shallow-water relatives can be useful as a means of inferring these features in related deep-sea species. In a recent paper describing gametogenesis, spawning behavior, and early development in another methane-seep polychaete, *Hesiocaeca methanicola* (Eckelbarger *et al.*, 2001), we applied "phylogenetic inference" as a means of predicting life-history features that are difficult to document directly. In his description of *Methanoaricia dendrobranchiata*, Blake (2000) stated that this species is difficult to place in any known polychaete family, but it has more affinities to the Orbiniidae than any other. The present study documents several reproductive features of *M. dendrobranchiata* that are shared with other orbiniids: (1) repeated ovaries in a large number of segments, (2) an ovary associated with a blood vessel nexus, (3) intraovarian oogenesis, (4) large, yolky eggs, (5) sperm developing as coelomic clones, and (6) mature spermatozoa with a similar centriolar satellite complex and subacrosomal material. On the basis of the life histories of other orbiniids (summarized in Table 1), we predict that *M. dendrobranchiata* is an annual, iteroparous breeder that produces an egg mass or cocoon.

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